PS 19
Calcium Signalling in Beta-Cells

475

ROLE OF THE SARCO-ENDOPLASMIC RETICULUM Ca^{2+}-ATPASE SERCA3 IN Ca^{2+} SIGNALING IN MOUSE β-CELLS.

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Pancreatic islets express both the high Ca^{2+}-affinity SERCA2b and the low Ca^{2+}-affinity SERCA3. Dysregulation of the SERCA3 activity has been associated with type 2 diabetes in mice and humans. Wild type (+/+) and SERCA3 KO (-/-) mice were used to evaluate the role of SERCA3 in β-cell $[Ca^{2+}]_c$ regulation and insulin secretion, and to estimate its contribution to glucose homeostasis. **Methods.** $[Ca^{2+}]_c$ was measured (fura-PE3) in isolated islets or clusters of islet cells, and insulin secretion was monitored from perifused islets. **Results.** Stimulation with 15 mmol/l glucose (G) induced an initial, transient drop in $[Ca^{2+}]_c$ ascribed to pumping into the endoplasmic reticulum (ER) because of its suppression by thapsigargin (TG). This drop was unaffected by SERCA3 ablation. Mobilization of intracellular Ca^{2+} by acetylcholine or TG was similar in +/+ and -/- β-cells. $[Ca^{2+}]_c$ oscillations induced by glucose or pulses of high K^+ normally display a slow descending phase caused by slow Ca^{2+} release from the ER. SERCA3 ablation (like TG) increased the peak of $[Ca^{2+}]_c$ during the oscillations and accelerated the descending phase. This indicates that Ca^{2+} released by the ER into the cytosol at the end of each oscillation was pumped into the ER by SERCA3 during the upstroke phase. Average $[Ca^{2+}]_c$ at 3 to 20 mmol/l G was similar in +/+ and -/- β-cells. Biphasic insulin secretion in response to 15 mmol/l was not smaller in -/- than +/+ islets. Refeeding after 24h fasting did not reveal impaired glucose homeostasis in -/- mice. **Conclusions** SERCA2b pumps Ca^{2+} into the ER at basal $[Ca^{2+}]_c$ in β-cells. SERCA3 becomes operative when $[Ca^{2+}]_c$ is elevated during $[Ca^{2+}]_c$ oscillations. SERCA3 expression is not required for normal insulin secretion and glucose homeostasis, and its defective expression does not contribute to development of type 2 diabetes as previously suggested.

476

Impaired transport of insulin secretory granules in the rat insulinoma cell line INS-1 expressing antisense Ca2+/calmodulin dependent protein kinase II d2 (CaM Kin II d2)

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Introduction: CaM Kin II d2 is supposed to play a role in stimulated insulin secretion. Synapsin I mediated actin web debundling after phosphorylation by CaM Kin II d2 is one mechanism thought to be involved in the regulated refilling of the so called readily releasable pool of insulin granules. We had shown a strong association between insulin secretory granules and CaM Kin II d2 by means of subcellular fractionation and immunofluorescence as well as coprecipitation of synapsin I and CaM Kin II d2 in previous works. An INS-1 cell line overexpressing antisense CaM Kin II d2 showed a decrease in CaM Kin II expression.
Aim: It was the aim of this study to investigate the distribution of insulin granules in insulinoma cells overexpressing CaM Kin II d2 by confocal microscopy.
Methods: INS-1 cells overexpressing antisense CaM Kin II d2 were grown in RPMI 1640 medium under standard conditions. Confocal laser immunofluorescence was done with a Zeiss laser scanning microscope. Visualization was performed with a anti-insulin antibody and anti-mouse secondary antibody purchased from sigma
Results: Confocal laser immunofluorescence of antisense cells showed a pattern of insulin granule distribution distinct from wt INS-1 cells. In contrast to the regular submembraneous pattern of insulin secretory granules in INS-1 there was a perinuclear pattern of insulin secretion granules in CaM Kin II d2 antisense expressing cells.
Discussion: These findings point toward a role of CaM Kin II d2 in the modulation of insulin granule transport to the plasma membrane. The transport defect may be the consequence of decreased synapsin1 phosphorylation due to CaM Kin II d2 antisense expression.

477

THE HUMAN PEPTIDE, alpha-ENDOSULFINE, DIRECTLY BLOCKS L-TYPE CA2+ CURRENTS IN MIN6 ß-CELLS.

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Background and Aims: alpha-Endosulfine, a 121-amino acids 13-kDa protein which belongs to the cAMP-regulated-phosphoprotein family, was originally isolated as an endogenous ligand for the sulfonylurea receptor. alpha-Endosulfine has been shown to displace sulfonylurea binding, block ATP-sensitive K+-channels activity (KATP) and stimulate insulin secretion at basal glucose concentrations. At stimulatory glucose concentration, alpha-endosulfine inhibits insulin secretion and calcium influx. This inhibition, independent of KATP blockade, is observed on MIN6 ß-cells and isolated perfused rat pancreas. We have investigated the electrophysiological mechanism underlying this inhibitory effect.
Materials and Methods: Using the perforated-patch whole-cell patch-clamp technique, we have recorded on MIN6 cells the effects of alpha-endosulfine on membrane potential and calcium currents. Each value is quoted as mean± s.e.m.
Results: In the absence of glucose, the membrane potential (Vm) of the MIN6 cell was electrically silent at –67±1 mV (n=16). Under these conditions, the addition of 1 µM alpha-endosulfine resulted in a rapid depolarization of the membrane potential to a steady-state value of –19±6 mV (n=3) and a transient firing of action potentials. The presence of 10 mM glucose depolarized the MIN6 membrane potential to –51±2 mV and induced action potential firing. Subsequent addition of 1 µM alpha-endosulfine further depolarized Vm to –20±3.9 mV (n=7); this was associated with the abolition of action potentials. These effects were entirely reversed on removal of the peptide. Similar experiments performed with 200 µM tolbutamide showed that the sulfonylurea did not mimic the effect of alpha-endosulfine, but instead produced a small depolarization which was associated with a maintained stimulation of electrical activity. Under voltage-clamp, characteristic inward Ca2+-currents flowing through L-type Ca2+-channels were elicited at potentials positive to –60 mV from holding potential of –70 mV. At 0 mV, 1 µM alpha-endosulfine blocked the peak current by 40±2.3% and the steady-state current by 85±4% (n=4). The block was independent of both use and voltage.
Conclusions: The primary event occurring upon addition of alpha-endosulfine to MIN6 cells subjected to glucose stimulation, is inhibition of L-type Ca2+-channels. alpha-Endosulfine acts as an open Ca2+-channel blocker reducing Ca2+ influx and consequently insulin secretion.

478

Palmitate potentiates glucose-induced insulin secretion by increasing cytoplasmic Ca2+ and electrical activity in mouse B-cells

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Background and Aims: It is well established that acute exposure of pancreatic islets to free fatty acids leads to stimulation of insulin release. We have correlated the effects of palmitate on insulin secretion to changes of the cytoplasmic free Ca2+ ([Ca2+]i) with the goal of identifying the participating mechanisms.
Materials and Methods: Collagenase-isolated intact mouse pancreatic islets were used throughout. Changes in [Ca2+]i were recorded in fura-2 loaded islets using microfluorimetry. Insulin secretion was assessed by radioimmunoassay. Electrical activity was measured applying the perforated patch whole-cell configuration to B-cells in intact islets. Palmitate was applied at a concentration of 1 mM and was dissolved with 1% fatty acid free bovine serum albumin.
Results: Palmitate had no effect on basal insulin release measured at 3 mM glucose. Adding palmitate to islets exposed to 7.5 or 15 mM glucose increased insulin secretion by 40% and 85% respectively (n=8). The amount of insulin released in the simultaneous presence of 7.5 mM glucose and palmitate approached that obtained in response to 15 mM glucose alone. In islets exposed to 15 mM glucose, [Ca2+]i oscillated between a plateau level and discrete peaks. The frequency of these oscillations (peak-to-peak) was 2.2+0.58 min-1. Addition of 1 mM palmitate slightly (»15%) elevated the plateau concentration and increased the frequency of the oscillations to 3.2+0.46 min-1 (+45%). Occasionally (20%), addition of palmitate suppressed the oscillatory pattern and produced a sustained elevation of [Ca2+]i. [Ca2+]i rarely oscillated in islet exposed to the subthreshold glucose concentration 7.5 mM but oscillations similar to those seen at 15 mM glucose were then inducible by addition of palmitate. These effects of palmitate on [Ca2+]i and insulin secretion correlated with stimulation of glucose-induced (15 mM) electrical activity and the normal bursting pattern was replaced by uninterrupted action potential firing similar to what is observed at glucose concentrations ³20 mM.
Conclusions: We conclude that the ability of palmitate to stimulate insulin secretion involves stimulation of B-cell electrical activity and elevation of [Ca2+]i. We further propose that the stimulatory action is secondary to increased generation of ATP, which promotes insulin secretion by both closing KATP-channels (thus increasing electrical activity and Ca2+-entry) and providing the metabolic energy required for insulin exocytosis and/or mobilisation of new granules for release.